

# Episodes of water deprivation enhance daily hypertonic NaCl intake in rats

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## Abstract

Water and 1.8% NaCl intake was recorded daily in adult male rats (N = 6) submitted to four water deprivations plus four sodium appetite tests, each at the end of each 7-day interval, or in controls (non-deprived, N = 6). Water deprivation was achieved by removing water and 1.8% NaCl for 24 h. Water was then offered for 2 h. At the end of this period, 1.8% NaCl was also offered in addition to water (sodium appetite test). Average daily 1.8% NaCl intake was enhanced from  $5.2 \pm 1.0$  to  $15.7 \pm 2.5$  ml from the first to the fifth week in the experimental group and was unchanged in the control group. Daily water intake was not altered in either group. Thus, repeated episodes of water deprivation enhance daily NaCl intake.

## Key words

- Thirst
- Salt appetite
- Dehydration
- Natriuresis

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Mechanisms subserving need-induced sodium intake, i.e., the sodium intake that results from sodium depletion, have been studied in models of extracellular dehydration. The rat in these models becomes hypovolemic as a result of administration of intraperitoneal dialysis, hyperoncotic colloids or diuretics (1-4). Enhancement of need-induced sodium intake in the extracellular model is produced in the rat by a prior episode of sodium depletion (1-4). The episode of sodium depletion corresponds to a period of 24 h in negative sodium balance, after which normal hydration balance is restored by allowing the animal to ingest sodium and water (1-4). Daily need-free intake of hypertonic NaCl, i.e., sodium intake in the hydrated state, is also enhanced by episodes of sodium depletion (2,4).

Net sodium loss occurs during water deprivation as part of the mechanisms that blunt the increase in extracellular hypertonicity and consequent cell dehydration (5,6). Con-

current hypovolemia and activation of the renin-angiotensin system during water deprivation induce a specific appetite for sodium, clearly distinct from thirst (5-9). It is not known whether episodes of water deprivation also enhance sodium intake. The results of the present study show that daily sodium intake is enhanced by repeated episodes of water deprivation in rats.

Male Holtzman rats (280-300 g) were housed under standard laboratory conditions of a 12/12-h light-dark cycle,  $23 \pm 2^\circ\text{C}$  and  $55 \pm 10\%$  humidity. Regular food pellets (Purina chow), water and 1.8% NaCl solution were freely available for consumption unless otherwise stated. Daily intake of water and 1.8% NaCl was recorded using polypropylene tubes graduated to the nearest 1 ml and fitted with stainless steel spouts. Water deprivation and sodium appetite tests were performed as described (8). Briefly, water and 1.8% NaCl were removed from the cages and only food remained available

for 24 h. Then, food was removed and only water was given for 2 h in 0.1 ml graduated glass burettes fitted with stainless steel spouts. At the end of this 2-h period, 1.8% NaCl was

Figure 1. Daily intake of 1.8% NaCl (A) and water (B) by animals submitted to four episodes of water deprivation (deprived) or to no water deprivation at any time (not deprived). Arrows indicate the day of water deprivation. Episodes of water deprivation induced an increase in daily 1.8% NaCl intake which was different from that of not deprived controls (see text). Daily water intake was not altered by the episodes of water deprivation. \* $P < 0.05$  vs first block, prior to first deprivation.

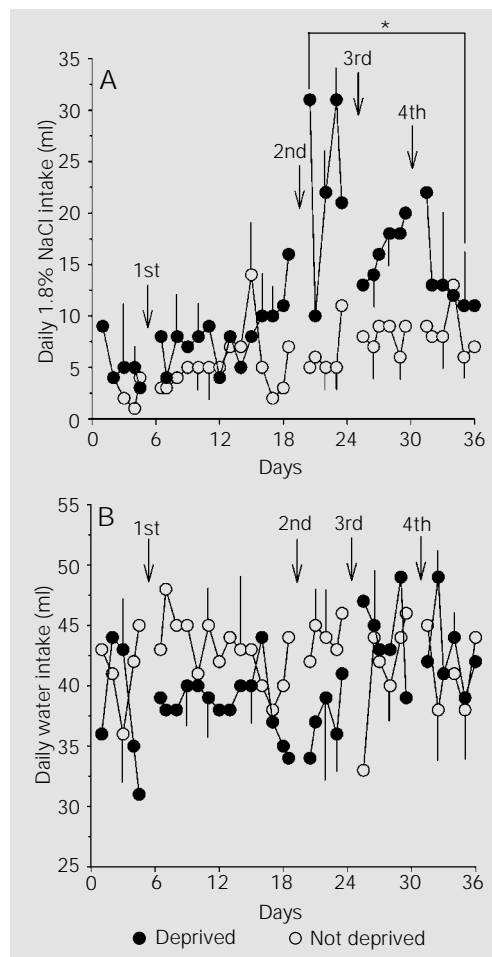
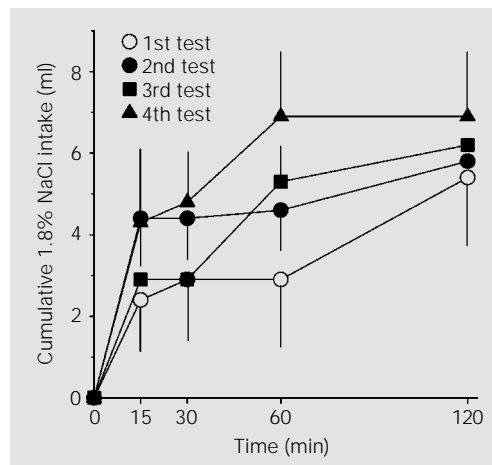


Figure 2. Cumulative 1.8% NaCl intake during repeated sodium appetite tests. There was no significant difference between the different appetite tests.



made available in glass burettes and ingestion of water and 1.8% NaCl was recorded at 15, 30, 60 and 120 min (sodium appetite test) and 24 h. Food was returned at the end of the 120-min period of the appetite test. Animals were submitted to four water deprivations and respective sodium appetite tests. Each water deprivation-sodium appetite test was repeated at a minimum interval of 7 days and a maximum interval of 14 days. Data for analysis of daily intake were organized into five blocks delimited by the four deprivations. Each block consisted of the averaged daily intake of each animal within that block. Data are reported as means  $\pm$  SEM. One- or two-way repeated or non-repeated measures analysis of variance (ANOVA) was used where applicable. Significance was set at  $P < 0.05$ .

Daily intake of 1.8% NaCl over 5 weeks of recording was enhanced in the group repeatedly deprived of water [ $F(4,20) = 6.1$ ,  $P < 0.05$ , one-way repeated measures ANOVA], and the intake of 1.8% NaCl of this group was higher than the intake of non-deprived controls [ $F(4,11) = 3.4$ ,  $P < 0.05$ , two-way ANOVA] (Figure 1A). Daily water intake was not altered by repeated water deprivation (Figure 1B). There were no statistical differences in sodium intake from the first to the fourth sodium appetite test [ $F(3,45) = 0.6$ , two-way repeated measures ANOVA] (Figure 2). Water intake during the sodium appetite test was not altered by repeated water deprivations ( $5.4 \pm 2.2$ ,  $5.8 \pm 1.6$ ,  $6.2 \pm 0.9$  and  $6.9 \pm 1.5$  ml/120 min, from the 1st to the 4th test, respectively;  $N = 6$ ).

The present results show that daily intake of 1.8% NaCl was enhanced by prior episodes of water deprivation. This is the same type of result that has been obtained with rats using the extracellular model of dehydration with either the diuretic furosemide (3,4) or hyperoncotic colloid (2). Experience with the ingestion of NaCl solution and persistent alterations in water-electrolyte balance after sodium depletion seem not to be important

for this enhancement (1-4), suggesting that a print in the brain generated by signals from sodium depletion tells the animal to ingest more NaCl solution when it becomes available. This print is likely to be related to the central nucleus of the amygdala, since lesion of this area almost completely reduces NaCl intake and delays the enhancement of daily sodium intake induced by sodium depletion (9). The enhancement is, however, not related to the anteroventral periventricular tissue surrounding the third ventricle, an area important for the angiotensin II-induced component of sodium intake, since lesion of this area does not alter either daily NaCl intake or its enhancement (10). The print might be related to organizational changes in the brain because pregnancy and neonatal sodium depletion or hypovolemia produce offspring with enhanced need-free intake during adulthood (11-13).

The failure to obtain significant enhancement of need-induced NaCl intake with repeated water deprivations contrasts with the experiments of extracellular models in which need-induced intake is clearly enhanced by only one prior episode of sodium depletion (1,3,9,10).

The enhancement of daily NaCl intake seems to be a phenomenon common to dif-

ferent models of sodium intake and it is probably an adaptation to recurrent sodium loss. Nonetheless, it is noteworthy that water intake is not altered by repeated episodes of dehydration in any of the models so far tested. This suggests a fundamental difference between thirst and salt appetite in the adaptive responses to repeated dehydration. In this regard, a goal for future experiments is to determine whether the enhancement could also occur at isotonic NaCl concentration since its ingestion by dehydrated rats is sometimes related to thirst and not to sodium appetite (13,14).

The water deprivation model is appropriate to study the mechanisms of sodium intake since it simulates what occurs in nature. Thus, the enhancement of sodium intake by water deprivation has implications for both the understanding of the mechanisms of sodium appetite and the management of hydration strategies directed at human and animal health (15,16).

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